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A Synthetic Study of (–)-Dihydroedulan II and Related Compounds†

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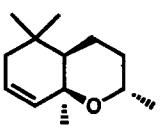
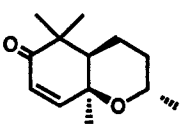
Abstract: (-)-Dihydroedulan II and related compounds were synthesized from (2*R*,1'*RS*)-dihydro-α-ionol, which was prepared by lipase-catalyzed enantioselective transesterification of the corresponding diastereomeric mixture as the key-step Fungal allylic oxidation of (-)-dihydroedulan II worked well to afford a corresponding alcohol as well as (+)-dihydroedulan-8-one

Introduction

Dihydroedulan II (**1**) and related compounds (**2**, **3a**) are degraded carotenoids, which have been isolated as volatile components from the hairpencils of butterflies, *Euploea klugii* (for **1** and **3a**)[1] and *Danaus plexippus* (for **2**) [2] The isolation of dihydroedulan II (**1**) and its diastereomer from the juice of *Passiflora edulis*[3] and the herb[4,5] was also reported Since these compounds are the trace components of the whole volatiles, few synthetic studies have been reported so far [1-3] Francke and coworkers[1] determined the absolute configuration of **3a** as depicted in Fig 1 However, the absolute configurations of other compounds are left unknown Since it is supposed that these compounds have close relationships with each other, the common absolute configuration for carbon skeleton is assumed to be the same Here we report the synthetic study of these compounds in optically active forms, which would clarify the stereochemistry of natural products as well as their physiological function in insects



(-)-Dihydroedulan II **1**



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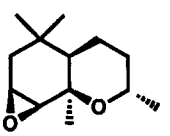


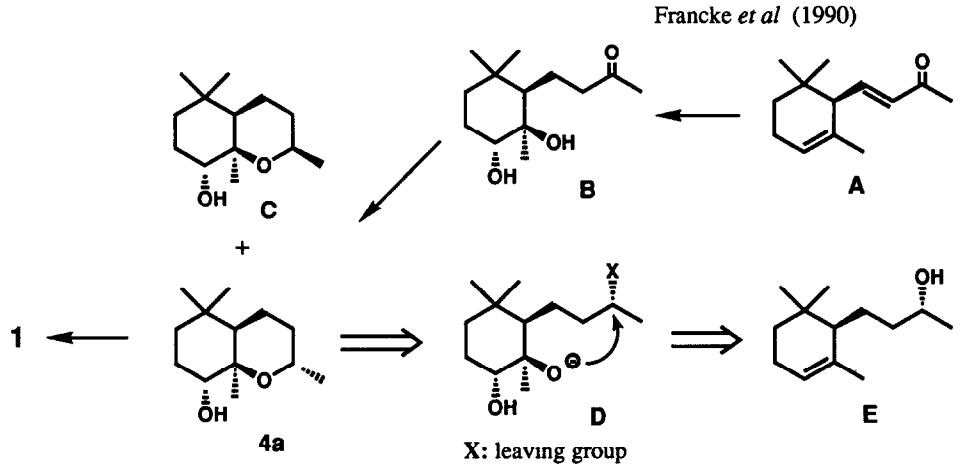
3a

Fig. 1. Dihydroedulan II and Related Compounds

† Preparation of Enantiomerically Enriched Compounds by Using Enzymes, Part 13 For Part 12, Sugai, T , Watanabe, N , Ohta, H *Tetrahedron Asymmetry*, 1991, *2*, 371 The experimental part was taken from the B Sc thesis of T Y (March, 1991)

Synthetic Plan

As mentioned above, Francke and co-workers accomplished the synthesis of optically active forms of these dihydroedulan derivatives starting from (*R*)-α-ionone (**A**) [1] The key-intermediate **4a** and its epimer (**C**), resulted from the cyclization of diol **B**, could be separated each other and **4a** was converted to final products (scheme 1) However, since the major interest of their synthesis was the structural determination of natural products, the *e es* of the final products were as low as *ca* 20%, reflecting the *e e* of the starting material Our synthetic plan is also shown in scheme 1 If the cyclization of diol intermediate (**D**) possessing a suitable leaving group (=X) takes place in an S_N2 fashion, it is expected that Francke's key-intermediate **4a** would be efficiently obtained Our recent studies on "preparation of enantiomerically enriched compounds by using enzymes" were planned to be applied for the preparation of the starting material, (2*R*,1'*R*) dihydro-α-ionol (**E**)

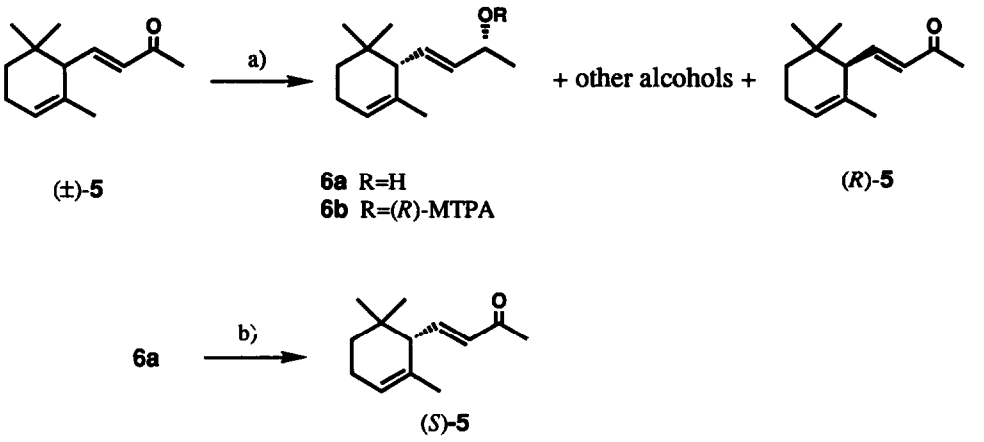


Scheme 1. Synthetic Plan

Attempted Biochemical Reduction of (±)-α-Ionone

The first attempt was the yeast reduction of commercially available (±)-α-ionone (**5**) It is well known that the stereochemical course of the reduction of simple ketone by yeasts generally obey the "Prelog's rule" [6] In contrast, *Pichia miso* IAM 4682 have been revealed to afford (*R*)-alcohol when a simple alkenyl methyl ketone was reduced [7] This strain was therefore applied expecting that (2*R*)-α-ionol (**6a**) having the desired stereochemistry would be obtained The reduction of (±)-**5** was achieved by using cells in stationary phase to give an inseparable mixture of alcohols (15%) The ratio of the diastereomers was revealed to be *ca* 77 9 9 5, by the 400 MHz ^{1}H NMR spectrum of the corresponding (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA)[8] ester The absolute configuration of the resulting asymmetric carbon (C-2 position) of the major isomer was estimated to be *R*, by the chemical shift of $\underline{CH_3}CH(OMTPA)$- proton compared to the previous examples[7,9] (see Experimental) The absolute configuration of another asymmetric center, existing on the six membered ring (C-1') of the alcohol was determined after oxidation to α-ionone To our disappointment, the present sample of α-ionone showed its specific rotation -220° (ethanol) which indicated that it was the (*S*)-isomer of 55% *o p* [(*R*)-isomer +421° (ethanol)] [10] This implies that a kinetic resolution

took place during the reduction, distinguishing the rather remote asymmetric center from carbonyl group. The absolute configuration of recovered ketone after yeast reduction was *R* as expected. However, it was rather difficult to achieve sufficient conversion to obtain (*R*)-**5** of high *o p*



a) *Pichia miso* IAM 4682 b) MnO_2/Et_2O

Scheme 2. Attempted Biochemical Reduction of (±)-α-Ionone

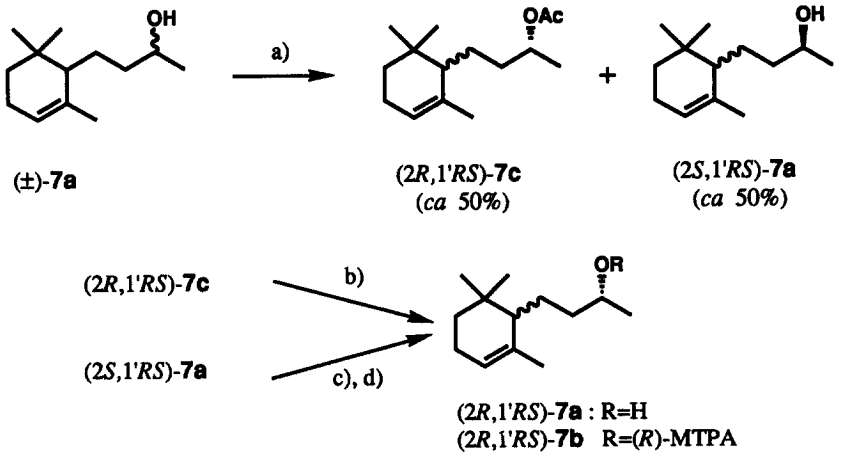
At this point, we turned our attention to the preparation of (2*R*,1'*RS*)-dihydro-α-ionol instead of (2*R*,1'*R*)-isomer, since the unwanted diastereomer (*ent*-C) derived from (2*R*,1'*S*)-isomer could be removed at the later stage

Preparation of (2R,1'RS)-Dihydro-α-ionol by Using Lipase

The preparation of (2*R*,1'*RS*)-dihydro-α-ionol (**7a**) was successfully achieved by lipase-catalyzed enantioselective transesterification[cf 11,12] as the key-step (scheme 3) When a mixture of (2*R*,1'*RS*)- and (2*S*,1'*RS*)-**7a** was treated with lipase PS (Amano, from *Pseudomonas*) and vinyl acetate (22°C, 24 h), recovered alcohol (*ca* 50%) and acetate **7c** (*ca* 50%) were obtained The former was revealed to be an almost 1 1 mixture of two major diastereomers by ^{1}H NMR analysis of the corresponding (*R*)-MTPA ester **7b** Again judged from the chemical shift of $\underline{CH_3}CH$(OMTPA)- proton (see Experimental), the alcohols were assigned to be (2*S*,1'*R*)- and (2*S*,1'*S*)-isomers This assumption is consistent with the empirical rule that in the acylation of simple methyl alkyl (alkenyl) carbinols, *Pseudomonas* lipase catalyzes the reaction of (*R*)-enantiomer faster than that of (*S*)-enantiomer The absolute configuration of recovered alcohol was therefore tentatively assigned to be *S* The *e e* (*d e*) concerning C-2 was estimated to be 94% by comparing geminal dimethyl (C-6') proton due to two major diastereomers and two minor diastereomers (97 3) On the other hand, the acetate thus obtained, consisted of (2*R*,1'*R*)- and (2*R*,1'*S*)-isomer, judged from its ^{1}H NMR spectrum of the corresponding (*R*)-MTPA ester, which was prepared *via* methanolysis and subsequent MTPA esterification The *e e* (*d e*) concerning C-2 was 96%

Next, transformation of both of the resolved products into dihydro-α-ionol possessing desired configuration was carried out Hydrolysis of the acetate **7c** to **7a** was performed in buffer solution by using

lipase PS, which had been employed for selective transesterification as already described, expecting further enantiomeric enhancement during hydrolysis by leaving the (*S*)-acetate intact. In practice, the hydrolysis proceeded smoothly to afford alcohol (2*R*,1'*RS*)-**7a** in 96% yield, of which *e e* (*d e*) was almost the same as that of the starting material The asymmetric center at C-2 of unreacted alcohol (2*S*,1'*RS*)-**7a** was smoothly inverted by Mitsunobu reaction[13] by using 3,5-dinitrobenzoic acid [14] The inverted alcohol (2*R*,1'*RS*)-**7a** was obtained (93%, 2 steps) after methanolysis, and exhibited 94% *e e* (*d e*) concerning to C-2 position



a) vinyl acetate, lipase PS b) lipase PS, phospnate buffer; 96%
c) DEAD, 3,5-DNBOH, Ph_3P/THF, quant
d) K_2CO_3/MeOH, H_2O, 93%(2steps)

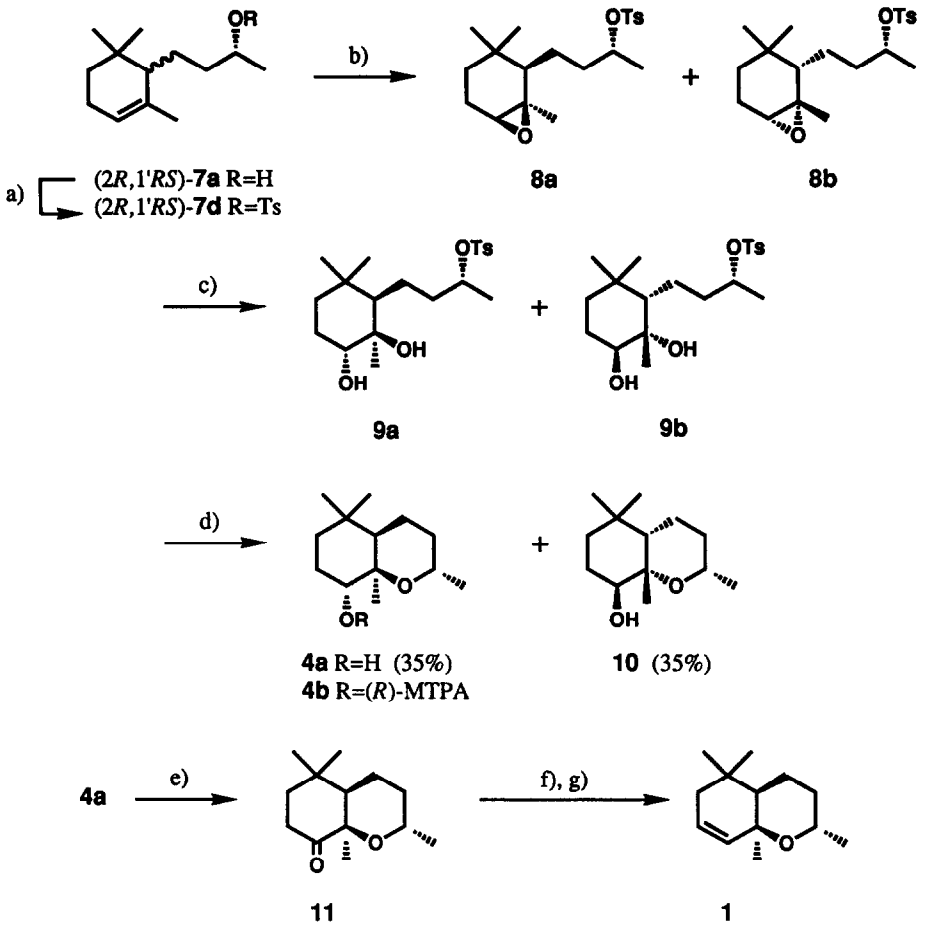
Scheme 3. Preparation of Starting Material

The alcohol possessing desired configuration (2*R*,1'*RS*)-**7a** thus obtained was combined [96% *e e* (*d e*) at C-2] in 68% yield from α-ionone, and employed for the subsequent step The whole procedures already described could be performed by using over 15 g of the starting material, (2*RS*,1'*RS*)-**7a.**

Synthesis of Key-intermediate 4a and Dihydroedulan II

Diol intermediate **9a** and **9b**, the precursor for cyclization, was prepared according to the Francke's procedure with some modifications Thus, tosylate (**7d**, 93%) was epoxidized by using monoperoxyphthalic acid monomagnesium salt (MMPP) to afford epoxide [**8a** + **8b**, 64%] whose oxygen atom was introduced onto the same face of six-membered ring with the side chain [15] Perchloric acid-catalyzed epoxide opening reaction in a diaxial manner[1] gave a mixture of **9a** + **9b** (60%) As separation of any diastereomeric intermediates so far obtained was difficult, the mixture of **9a** + **9b** was employed for cyclization

At this point, the conditions for cyclization were optimized concerning solvent, base and reaction temperature, by using corresponding racemate From those results, the best selection was proven to be the use of sodium hydride (3 5 eq) in refluxing tetrahydrofuran (THF), to afford **4a** and its diastereomer **10** in the highest yield In the case that optically active forms of **9a** + **9b** were used, **4a** (35%) and **10** (35%) were obtained after chromatographic separation and subsequent recrystallization



a) TsCl/Py, CH_2Cl_2, 94% b) MMPP/*i*-PrOH, H_2O, 64%
c) $HClO_4$/THF, H_2O, 60% d) NaH/THF
e) CrO_3, H_2SO_4, H_2O/acetone, 96% f) Tosylhydorazine/aqMeOH
g) NaH/toluene, 37% from **11**

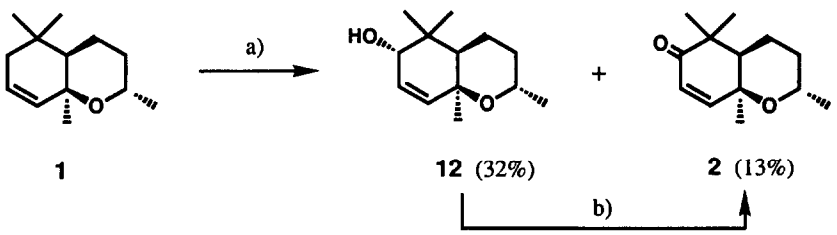
Scheme 4. Synthesis of Dihydroedulan II

NMR spectrum of **4a** was coincided with that reported by Francke *et al* [1] Its $[\alpha]_D$ value [-1 3° (ethanol)], however, was not in good accordance with the one estimated from Francke's value [-1 0° (ethanol) for *ca* 20% *e e*] The *e e* of our sample was confirmed to be over 99%, judging from NMR spectrum of its (*R*)-MTPA ester **4b.** This fact shows that the cyclization proceeded *via* complete S_N2 fashion Since the sign of the rotation for our sample was minus and coincided with Francke's one, the assumption for the absolute configuration of the starting material was proved to be correct This was supported by the fact that our diastereomeric alcohol **10** [$[\alpha]_D$ +8 7° (ethanol)] was in relation to the enantiomer of Francke's sample [$[\alpha]_D$ -1 5°]

The alcohol **4a** was oxidized to ketone **11** (96%) by CrO_3-H_2SO_4 This was also a crystalline material and could be purified by recrystallization. Bamford-Stevens reaction[1] of the corresponding tosylhydrazone afforded dihydroedulan II [**1**, 37%, 2 steps, $[\alpha]_D$ -26 2° (pentane)] after chromatographic purification followed by distillation (GLC single peak)

Synthesis of (+)-Dihydroedulan-8-one

The next task was the introduction of oxygen atom to dihydroedulan II (**1**) Francke *et al* employed t-butylhydroperoxide and chromium hexacarbonyl for the allylic oxidation [1] They could obtain dihydroedulan-8-one (**2**), but the yield and the specific rotation of the product were not described We planned to apply fungal oxidation system for the allylic oxidation, which had recently been developed for the functionalization of ionones [16] During the course of screening for the oxidation of α-ionone, a strain of *Aspergillus niger* was found as the most potent microorganism To know the scope and limitation of this microbial oxidation, (-)-dihydroedulan II (**1**) was incubated with the fungus Fortunately, it accepted this substrate, to afford alcohol **12** (32%) as well as dihydroedulan-8-one (**2**, 13%), although the yield was not so high probably because of the volatility of the substrate The orientation of newly introduced hydroxyl group in **12** was assigned to be α (8*S*), by NOE measurement (see Experimental) The stereochemistry is agreeable with the empirical rule derived from the study on the biochemical oxidation of structurally related cyclohexane derivatives [17] Alcohol **12** was cleanly oxidized with MnO_2 to afford **2** (73%) [$[\alpha]_D^{22}$ +20 8° (pentane)] Its NMR spectrum was in good accord with the copy of the spectrum kindly supplied by Prof Francke ††



a) *Aspergillus niger* b) MnO_2/Et_2O, 73%

Scheme 5. Microbial Oxidation of (-)-Dihydroedulan II

In conclusion, volatile components from the hairpencils of butterflies, Dihydroedulan II (**1**, *Euploea klugii*) and dihydroedulan-8-one (**2**, *Danaus plexippus*) were synthesized in optically active forms, by the combination of chemical and biochemical procedures

EXPERIMENTAL

All b ps and m ps were uncorrected IR spectra were measured as films for oils and KBr discs for solids on a Jasco IRA-202 spectrometer ^{1}H NMR spectra were measured in $CDCl_3$ with TMS as the internal standard at 90 MHz on a JEOL JNM FX-90 spectrometer or at 400 MHz on a JEOL JNM GX-400 spectrometer Optical rotations were recorded on a Jasco DIP 360 polarimeter Mass spectra were recorded on a Hitachi M-80 spectrometer at 70 eV Hitachi 163 gas chromatograph was used for GLC analysis Freshly distilled tetrahydrofuran (THF) from sodium-benzophenone ketyl, and distilled CH_2Cl_2 from CaH_2 were employed for anhydrous reaction Wako Gel B-5F and silica gel 60 K070-WH (70-230 mesh) of Katayama Chemical Co were used for prep TLC and column chromatography, respectively

†† Prof W Francke of Institut für Organische Chemie, Universität Hamburg, kindly informed us that the signal δ =1.09 published in their paper was a misprint, and must be corrected to δ = 1.13

Reduction of (±)-Ionone by Pichia miso IAM 4682 *Pichia miso* IAM 4682 was cultivated according to the reported procedure [7] The washed wet cells (115 g) were suspended in buffer solution (0 1M, 350 ml) To this was added glucose (35 g) and the mixture was shaken for 30 min (150 cps) at 30°C Then α-ionone (465 mg, 2 42 mmol) was added and the mixture was further shaken for 3 days The mixture was centrifuged (3500 rpm) and the supernatant was extracted with EtOAc after saturating with NaCl The precipitated cells were sonicated in acetone and filtered The filtrate was concentrated *in vacuo* and the residue was extracted with EtOAc Solid material on the filter was further extracted with EtOAc by applying sonication The organic extracts were combined and washed with water and brine, dried (Na_2SO_4) and concentrated *in vacuo* The residue was purified by SiO_2 flash column chromatography (6 g) Elution with hexane/EtOAc (10/1) afforded **6a** (66 mg, 15%), IR νmax 3350, 1140, 1060, 970, 950, 820 cm^{-1}, ^{1}H NMR δ 0 79 (3H, s), 0 88 (3H, s), 1 02-2 38 (6H, m), 1 28 (3H, d, *J*=7 5 Hz), 1 60 (3H, br s), 4 28 (1H, dq, *J*=5 9 Hz, 5 9 Hz), 5.20-5 68 (3H, m) A small portion was converted to the corresponding (*R*)-MTPA ester **6b**, ^{1}H NMR (400 MHz) δ 1 355 (3H, d, *J*=6 7 Hz, 9%), 1 365 (3H, d, *J*=6 7 Hz, 77%), 1 410 (3H, d, *J*=6 7 Hz, 5%), 1 420 (3H, d, *J*=6 7 Hz, 9%) An authentic specimen of **6b** prepared from (2*RS*,1'*RS*)-**6a** showed each signal in an equal amount The absolute configuration of the present sample concerning C-2 was assigned in the following manner $C\underline{H}_3CH[OMTPA(R)]CH_2R$ proton of two examples with known absolute configuration appears as shown below Ethyl (*S*)-3-hydroxybutanoate[9] (68%*e e* , R = $CO_2CH_2CH_3$) 1 33 [d, *J*=6 3 Hz, 16%, (*R*)], 1 42 [d, *J*=6 3 Hz, 84%, (*S*)] (*R*)-Sulcatol[7] [88% e e , R = $CH_2CH{=}C(CH_3)_2$] 1 26 [d, *J*=7 3 Hz, 94%, (*R*)], 1 35 [d, *J*=7 3 Hz, 6%, (*S*)] It is concluded that in both cases $C\underline{H}_3CH[OMTPA(R)]CH_2R$ signal due to (*R*)-isomer appeared in higher field than that of (*S*)-isomer Comparing these results, the major signal of **6b** [1 365 (3H, d, *J*=6 7 Hz, 77%)] must be due to either (2*R*,1'*R*)- or (2*R*,1'*S*)-isomer

To a soln of **6a** (31 1 mg, 0 16 mmol) in Et_2O (3 ml) was added MnO_2 (600 mg) and the mixture was stirred at room temp for 3 h The mixture was filtered through a pad of Celite and the filtrate was concentrated *in vacuo* The residue was purified by preparative TLC (hexane/EtOAc, 10/1) to give **5** (21 1 mg, 69%), $[\alpha]_D^{27}$ -220° (c=1 06, EtOH) [lit.[10] $[\alpha]_D^{22}$ +421° (c=0 636, EtOH) for (*R*)-**5**] Its IR and NMR spectra were identical with those of authentic (±)-α-ionone

When the yeast reduction of (±)-α-ionone was carried out for a prolonged incubation period, (*R*)-**5a** (40%) was recovered, $[\alpha]_D^{27}$ +20° (c=1 06, EtOH) Its *e e* was estimated to be 5% by the comparison of the specific rotation

4-(2',6',6'-Trimethyl-2'-cyclohexenyl)butan-2-ol (dihydro-α-ionol) **7a** A mixture of **5** (20 0 g, 0 10 mol), KOH (1 0 g, 17 8 mmol), 10% Pd-C (400 mg) in EtOH (500 ml) was stirred vigorously under H_2 for 3 h at room temp The mixture was filtered through a pad of Celite The filtrate was concentrated *in vacuo* to a half volume and to this was added $NaBH_4$ (2 4 g, 63 4 mmol) After stirring for 12 h at room temp, the mixture was neutralized with 1000 ml of 1 2N HCl and concentrated *in vacuo* The residue was extracted three times with Et_2O The extract was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo* Distillation of the residue afforded **7a** as diastereomeric mixture (14 6 g, 72%), b p 94°C/2 2 Torr; IR νmax 3360, 1130, 1080, 980, 940, 815 cm^{-1}, ^{1}H NMR δ 0 90 (3H, s), 0 94 (3H, s), 1 08-1 50 (8H, m), 1 20 (3H, d, J=5 9 Hz), 1 68 (3H, br s), 1 80-2 18 (2H, m), 3 50-3 85 (1H, m), 5 29-5 55 (1H, m)

(2R,1'RS)-4-(2',6',6'-trimethyl-2'-cyclohexenyl)but-2-yl acetate **7c** *and (2S,1'RS)-4-(2',6',6'-trimethyl-2'-cyclohexenyl)-2-butanol (dihydro-α-ionol)* **7a** To a soln of (±)-**7a** (9 62 g, 49 1 mmol) in vinyl acetate (270 ml) was added lipase PS (9 6 g), and the mixture was stirred for 24 h at 22°C The mixture was then filtered and the filtrate was concentrated *in vacuo* The residue was purified by SiO_2 flash column chromatography (460 g) Elution with hexane/EtOAc (10/1) afforded **7c** (5 76 g, *ca* 50%) as a diastereomer mixture, IR νmax 1740, 1240, 1130, 1070, 1020, 980, 950, 850, 810 cm^{-1}, ^{1}H NMR δ 0 87 (3H, s), 0 93 (3H, s), 0 90-2 10 (9H, m), 1 21 (3H, d, *J*=6 1 Hz), 1 68 (3H, br s), 2 04 (3H, s), 4 83 (1H, tq, *J*=5 7, 5 7 Hz), 5 30 (1H, m) Further elution with the same solvent system afforded **7a** (4 74 g, *ca* 50%) Its IR spectrum was identical with that of starting material

The *e es* of the samples above were determined as follows MTPA ester **7b** was prepared from the corresponding alcohol according to the reported procedure [8] **7b** from (2*RS*,1'*RS*)-(±)-**7a** ^{1}H NMR (400 MHz, $CDCl_3$) δ 0 790 (s), 0 810 (s), 0 815 (s), 0 825 (s), 0 850 (s), 0 855 (s), 0 865 (s), 0 875 (s) Each signal was shown in an equal amount In the case of **7b** prepared from (1*R*,1'*RS*)-**7c** *via* methanolysis and MTPA esterification, the ratio of the intensity of former four signals and that of latter four

signals was 2 98, therefore the *e e* of (2*R*,1'*RS*)-**7c** was determined to be 96% In the case of **7b** prepared from (2*S*,1'*RS*)-**7a**, the ratio of the intensity of former four signals and that of latter four signals was 97 3, therefore the *e e* of (2*S*,1'*RS*)-**7a** was confirmed to be 94% $CH_3CH(OMTPA)$ signal appeared at δ 1 262, 1 265 [each 1 5H, for (1*R*,1'*RS*)-**7b**] and 1 338 [3H, for (1*S*,1'*RS*)-**7b**]

(2R,1'RS)-4-(2',6',6'-Trimethyl-2'-cyclohexenyl)-2-butanol (dihydro-α-ionol) **7a** From (2*R*,1'*RS*)-**7c** A mixture of (2*R*,1'*RS*)-**7c** (5 76 g , 24 2 mmol) and lipase PS (6 g) in phosphate buffer (pH 7 0, 0 1 M, 600 ml) was shaken (*ca* 150 cpm) at 30°C for 20 h The mixture was extracted with EtOAc A small portion was analyzed by GLC (column, 15% BDS, 2 m, 60°C + 3°C/min, N_2, 0 8 kg/cm^2) rt 10 7 min (2 0%, **7c**), rt 12 0 min (98 0%, **7a**) The extract was washed with water, sat $NaHCO_3$ aq and brine, dried (Na_2SO_4) and concentrated *in vacuo* The residue was purified by SiO_2 flash column chromatography (400 g) Elution with hexane/EtOAc (10/1) afforded (2*R*,1'*RS*)-**7a** (4 57 g, 96%) Its IR and NMR spectrum were identical with those of (2*RS*,1'*RS*)-**7a** Its *e e* was determined to be 97% in the same manner as described above.

From (2*S*,1'*RS*)-**7b** According to the reported procedure,[14] a mixture of (2*S*,1'*RS*)-**7a** (5 56 g, 28 4 mmol), Ph_3P (14 9 g, 56 8 mmol), 3,5-dinitrobenzoic acid (12 0 g, 56 7 mmol) and diethyl azodicarboxylate (9 80 g, 56 3 mmol) in THF (150 ml) was stirred for 12 h at room temp Then the mixture was concentrated *in vacuo* The residue was purified by SiO_2 flash column chromatography (460 g) Elution with hexane/EtOAc (20/1) afforded the 3,5-dinitrobenzoate (11 6 g, quant), IR νmax 3110, 1735, 1630, 1645, 1280, 1170, 1130, 1075, 920, 855, 820, 775, 720 cm^{-1} This was dissolved in MeOH (300 ml) and K_2CO_3 (10 g) was added, then the mixture was stirred for 12 h at room temp Water was added to the mixture and the resulting mixture was stirred for further 6 h Then the mixture was concentrated *in vacuo* and the residue was extracted with EtOAc The extract was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo* The residue was purified by SiO_2 flash column chromatography (400 g) Elution with hexane/EtOAc (10/1) afforded (2*R*,1'*RS*)-**7a** (5 15 g, 93%, 2 steps) Its IR and NMR spectrum were identical with those of (2*RS*,1'*RS*)-**7a** Its *e e* was determined to be 94% in the same manner as described above

The *e e* of the combined (2*RS*,1'*RS*)-**7a** was determined to be 96% in the same manner

(1R,1'RS)-1-Methyl-3-(2',6',6'-trimethyl-2'-cyclohexenyl)propyl p-toluenesulfonate **7d.** A mixture of (2*RS*,1'*RS*)-**7a** (9 72 g, 49 6 mmol), TsCl (15 0 g, 78 7 mmol) and pyridine (17 ml) in CH_2Cl_2 (40 ml) was stirred under Ar with ice-cooling for 2 days The mixture was diluted with water and extracted with EtOAc The extract was washed with sat $CuSO_4$ aq, sat Na_2SO_4 aq and brine, dried (Na_2SO_4) and concentrated *in vacuo* The residue was purified by SiO_2 flash column chromatography (420 g) Elution with hexane/EtOAc (10/1) afforded **7d** (16 3g, 94%), IR νmax 1600, 1175, 1100, 890, 815, 780, 665, 575, 555 cm^{-1}, ^{1}H NMR δ 0 79 (6H, m), 1 05-1 61 (9H, m), 1 26 (3H, d, *J*=5 9 Hz), 1 59 (3H, br s), 2 42 (3H, s), 4 33-4 76 (1H, m), 5 16-5 41 (1H, m), 7 35 (2H, d, *J*=9 5 Hz), 7 80 (2H, d, *J*=9 5 Hz) This was employed for the next step without further purification

(1R,1'R,2'R*,3'S*)-3-(2',3'-Epoxy-2',6',6'-trimethyl-2'-cyclohexenyl)-1-methylpropyl p-toluenesulfonate* **8a** *and* **8b** A mixture of **7d** (16 3 g, 46 6 mmol), MMPP (140 g, 283 mmol) in *i*-PrOH (140 ml) and water (140 ml) was stirred at room temp for 12 h Then NaCl was added and the mixture was filtered The filtrate was extracted with EtOAc The extract was washed with water, sat $NaHCO_3$ aq and brine, dried (Na_2SO_4) and concentrated *in vacuo* The residue was purified by SiO_2 flash column chromatography (420 g) Elution with hexane/EtOAc (5/1) afforded a mixture of **8a** and **8b** (10 8 g, 64%), IR νmax 1600, 1180, 1100, 900, 820, 780, 740, 660, 580, 560 cm^{-1}, ^{1}H NMR δ 0 71 (3H, s), 0 89 (3H, s), 1 05-2 38 (12H, m), 1 29 (3H, m), 2 44 (3H, s), 2 90 (1H, br s), 4 32-4 89 (1H, m), 7 31 (2H, d, *J*=9 5 Hz), 7 84 (2H, d, *J*=9 5 Hz) This was employed for the next step without further purification In the preliminary experiment, a small amount (*ca* 3% of the major diastereomers) of other diastereoemers of epoxides judged by its ^{1}H NMR, was obtained by the futher elution of the chromatography

(1R,1'R,2'R*,3'R*)-3-(2',3'-Dihydroxy-2',6',6'-trimethyl-2'-cyclohexenyl)-1-methylpropyl p-toluenesulfonate* **9a** *and* **9b** To a soln of **8a** and **8b** (10 6 g, 28 9 mmol) in THF (80 ml) and water (80 ml) was added $HClO_4$ aq (37%, 8 ml) at room temp The mixture was stirred for 2 days Then powdered $NaHCO_3$ was added and the mixture was extracted with EtOAc The extract was washed with water, sat $NaHCO_3$ aq and brine, dried (Na_2SO_4) and concentrated *in vacuo* The residue was purified by SiO_2 flash column chromatography (420 g) Elution with hexane/EtOAc (1/1) afforded a mixture of **9a** and **9b** (6 7 g, 60%), IR νmax 3575, 1600,

1500, 1190, 1180, 1100, 1075, 960, 915, 900, 820, 660, 580, 555 cm^{-1}, ^1H NMR δ 0 75, 0 80, 0 85, 0.85 (total 6H, each s), 1 05-2 30 (9H, m), 1 15, 1 18 (total 3H, each s), 1 31 (3H, d, *J*=6 4 Hz), 2 42 (3H, s), 3 50 (1H, deformed dd, *J*=2 6, 2 6 Hz), 4 40-4 70 (1H, m), 7 36 (2H, d, *J*=8 7 Hz), 7 81 (2H, d, *J*=8 7 Hz) This was employed for the next step without further purification

(1R,3S,6R,10R)-(-)-1,3,7,7-Tetramethyl-2-oxabicyclo[4 4 0]decan-10-ol **4a** *and (1S,3S,6S,10S)-(+)-1,3,7,7-Tetramethyl-2-oxabicyclo[4 4 0]decan-10-ol* **10** A soln of **9a** and **9b** (6 70 g, 17 4 mmol) in THF (100 ml) was added dropwise to a stirred and ice-cooled suspension of NaH (2 5 g, 60% in mineral oil, 62 5 mmol, washed with hexane) in THF (50 ml) under Ar After stirring at 70°C for 2 h, the mixture was poured into ice-cooled sat NH_4Cl aq and extracted with EtOAc The extract was washed with water, sat $NaHCO_3$ aq and brine, dried (Na_2SO_4) and concentrated *in vacuo* The residue was purified by SiO_2 flash column chromatography (420 g) Elution with hexane/EtOAc (10/1) afforded **4a**. This was recrystallized from hexane to give colorless needles (1 31 g, 35%), m p 55-56°C (lit.[1] m p 58-59°C), $[\alpha]_D^{25}$ -1 3° (c=2 07, EtOH) [lit.[1] $[\alpha]_D^{22}$ -1 0° (c=0 291, EtOH)], IR vmax 3480, 2980, 2950, 2880, 1450, 1380, 1375, 1350, 1300, 1260, 1195, 1185, 1120, 1095, 1075, 1045, 985, 970, 940, 920, 870, 845 cm^{-1}, ^1H NMR (400 MHz, C_6D_6) δ 0 68 (3H, s), 0 96 (3H, s), 0 96-1 88 (8H, m), 1 08 (3H, d, *J*=5 9 Hz), 1 19 (1H, m), 1 43 (3H, s), 1 81 (1H, m), 3 42 (1H, m), 4 01 (1H, dd, *J*=4 9, 11 7 Hz) (Found. C, 73 23, H, 11 13 Calc for $C_{13}H_{24}O_2$ C, 73 54, H, 11 39%) Its IR and NMR spectra were identical with those reported previously [1] This was converted into MTPA ester **4b** in a usual manner [8] ^1H NMR (400 MHz, $CDCl_3$) δ 3 61 (3H, s) An authentic specimen **4b** prepared from (±)-**4a** δ 3 56 (1 5H, s), 3 61 (1 5H, s) Therefore (-)-**4a** was revealed to be over 99% *e e*

Further elution afforded **10**. This was recrystallized from hexane to give colorless needles (1 31 g, 35%), m p 77-78°C (lit.[1] m p 57-58°C for *ent*-**10**), $[\alpha]_D^{25}$ +8 7° (c=0 39, EtOH) [lit.[1] $[\alpha]_D^{22}$ -1 5° (c=0 336, EtOH) for *ent*-**10**], IR vmax 3440, 2980, 2940, 2900, 1475, 1450, 1380, 1360, 1240, 1220, 1160, 1140, 1100, 1075, 1060, 1000, 980, 970, 910, 850, 820 cm^{-1}, ^1H NMR (400 MHz, C_6D_6) δ 0 92 (3H, s), 1 10 (3H, s), 1 11 (3H, d, *J*=5 9 Hz), 1 23 (1H, dd, *J*=3 4, 5 9 Hz), 1 36 (3H, s), 1 06-1 88 (8H, m), 2 17 (1H, m), 3 63 (1H, dd, *J*=2 9, 3 9 Hz), 3 80 (1H, m) Its IR and NMR spectra were identical with those reported previously for *ent*-**10** [1] (Found C, 73 33, H, 11 18 Calc for $C_{13}H_{24}O_2$ C, 73 54, H, 11 39%)

(1R,3S,6R)-(-)-1,3,7,7-Tetramethyl-2-oxabicyclo[4 4 0]decan-10-one **11** To a soln of **10** (936 1 mg, 4 46 mmol) in acetone (30 ml) was added dropwise a soln of CrO_3 (3 0 ml, 2M in 6N H_2SO_4) with stirring and ice-cooling The mixture was stirred at room temp for 30 min After decomposing excess CrO_3 by addition of *i*-PrOH, the mixture was concentrated *in vacuo* The residue was extracted with EtOAc The extract was washed with water, sat $NaHCO_3$ aq and brine, dried (Na_2SO_4) and concentrated *in vacuo* The residue was purified by SiO_2 flash column chromatography (70 g) Elution with hexane/EtOAc (10/1) afforded **11**. This was recrystallized from hexane to give colorless prisms (886 mg, 96%), m p 50°C, $[\alpha]_D^{20}$ -25 8° (c=1 05, EtOH) [lit [1] $[\alpha]_D^{22}$ -7 7° (c=0 727, EtOH)], IR vmax 1715, 1490, 1180, 1145, 1115, 1090, 1070, 1050, 1005, 870, 850, 820, 760, 650, 610, 560 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ 1 01 (3H, s), 1 16 (3H, d, *J*=6 4 Hz), 1 13-1 22 (2H, m), 1 37 (3H, s), 1 54 (3H, s), 1 56-1 67 (3H, m), 1 80-1 94 (2H, m), 2 25 (1H, ddd, *J*=2 9, 4 9, 15 6 Hz), 2 65 (1H, ddd, *J*=6 8, 13 7, 15 6 Hz), 3 69 (1H, m) (Found C, 74 01, H, 9 72 Calc for $C_{13}H_{22}O_2$ C, 74 24, H, 10 54%)

(1S,3S,6R)-(-)-1,3,7,7-Tetramethyl-2-oxabicyclo[4 4 0]dec-9-ene (dihydroedulan II) **1** According to the reported procedure,[1] ketone **11** (886 mg, 4 21 mmol) was converted to (-)-**1** (301 mg, 37%), b p 145°C/2 Torr (bulb-to-bulb distillation), $[\alpha]_D^{25}$ -26 2° (c=1 12, pentane) [lit [1] $[\alpha]_D$ +0 6° (c=1 04, pentane)], IR vmax 3040, 2980, 2950, 2880, 1440, 1390, 1370, 1340, 1280, 1230, 110, 1095, 1070, 1020, 1000, 830, 730 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ 0 95 (3H, s), 1 03-1 31 (4H, m), 1 07 (3H, s), 1 10 (3H, d, *J*=6 4 Hz), 1 38 (3H, s), 1 46-1 64 (1H, m), 1 76-1 83 (1H, m), 1 96 (1H, br d, *J*=18 5 Hz), 3 32 (1H, m), 5 42 (1H, d, *J*=10 3 Hz), 5 67 (1H, ddd, *J*=2 4, 5 4, 10 3 Hz), ^1H NMR (400 MHz, C_6D_6) δ 0 80 (3H, s), 0 98 (3H, s), 1 03-1 22 (3H, m), 1 19 (3H, d, J=5 9 Hz), 1 34-1 45 (2H, m), 1 53 (3H, s), 1 59-1 65 (1H, m), 1 78-1 83 (1H, br d, J=18 1 Hz), 3 38 (1H, m), 5 52-5 59 (2H, m) Its IR and NMR (in C_6D_6) spectra were identical with those reported previously for **1** [1] MS *m/z* 194 [18%, (M^+)], 179 (100%), 161 (4%), 119 (22%), 107 (44%), 69 (40%), 40 (25%) MS *m/z* 194 1678 Calc for $C_{13}H_{22}O$ 194 1669 GLC (column, 15% BDS, 2 m, 80°C + 3°C/min, N_2, 0 8 kg/cm^2) rt 7 0 min (single peak)

(1S,3S,6R)-(+)-1,3,7,7-Tetramethyl-2-oxabicyclo[4 4 0]dec-9-en-8-one (dihydroedulan-8-one) **2** *and (1S,3S,6R,8S)-(+)-1,3,7,7-tetramethyl-2-oxabicyclo[4 4 0]dec-9-en-8-ol* **12** (-)-Dihydroedulan II (total 127 4 mg, 0 664 mmol, each *ca* 20 mg) was added to a culture broth of *Aspergillus niger*[16] (100 ml x 7 flasks) and the flasks were shaken on a gyrorotary shaker (180 rpm) for 2 days at 30°C The mixture was filtered through a pad of Celite Both the filtrate and residue on the filter was extracted with EtOAc The combined extract was washed with brine, dried (Na_2SO_4) and concentrated *in vacuo* The residue was purified by preparative TLC (hexane/EtOAc, 2/1) to give **2** (18 0 mg, 13%) and **12** (44 4 mg, 32%)

2 $[\alpha]_D^{22}$ +20 1° (c=0 90, pentane), IR vmax 3040, 2980, 2950, 2880, 1680, 1480, 1440, 1385, 1365, 1230, 1110, 1080, 1065, 990, 825 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ 1 13 (3H, s), 1 14 (3H, d, *J*=6 0 Hz), 1 16-1 95 (5H, m), 1 32 (3H, s), 1 58 (3H, s), 3 37-3 50 (1H, m), 5 94 (1H, d, *J*=10 2 Hz), 6 55 (1H, dd, *J*=2 0, 10 2 Hz), MS *m/z* 194 1288 Calc for $C_{13}H_{20}O_2$-CH_4 194 1305 (Found C, 74 82, H, 9 43 Calc for $C_{13}H_{20}O_2$ C, 74 96, H, 9 68%)

12 $[\alpha]_D^{23}$ +36 6° (c=0 77, EtOH), IR vmax 3460, 3040, 2980, 2950, 2880, 1680, 1450, 1440, 1380, 1370, 1280, 1250, 1230, 1160, 1130, 1080, 1075, 1050, 1030, 1020, 995, 875, 825, 745, 660 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ 1 07 (3H, s), 1 10 (3H, s), 1 11 (3H, d, *J*=6 4 Hz), 1 17-1 32 (3H, m), 1 40 (3H, s), 1 42-1 65 (3H, m), 1 85-1 94 (1H, m), 3 28-3 37 (1H, m), 4 13 (1H, dd, *J*=0 5, 2 0 Hz), 5 48 (1H, ddd, *J*=2 4, 2 4, 10 3 Hz), 5 63 (1H, dd, *J*=2 0, 10 3 Hz), NOE (400 MHz, $CDCl_3$) H_{C8} irradiation H_{C5} 7 3%, $H_{C7\ \beta Me}$ 3 8% gain, $H_{C1\text{-}Me}$ irradiation $H_{C7\text{-}\alpha Me}$ 9 1% gain MS *m/z* 196 1475 Calc for $C_{13}H_{22}O_2$-CH_4 196 1463

(1S,3S,6R)-(+)-1,3,7,7-Tetramethyl-2-oxabicyclo[4 4 0]dec-9-en-8-one (dihydroedulan-8-one) **2** A mixture of **12** (19 mg, 0 0856 mmol) and MnO_2 (600 mg) in Et_2O (3 ml) was stirred at room temp for 12 h The mixture was filtered through a pad of Celite and the filtrate was concentrated *in vacuo* The residue was purified by preparative TLC (hexane/EtOAc, 10/1) to give **2** (13 0 mg, 73%), $[\alpha]_D^{22}$ +20 8° (c=1 30, pentane) Its IR and NMR spectra were identical with those already obtained

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